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APPLICATION NUMBER: *60/709,869*
FILING DATE: *August 19, 2005*
RELATED PCT APPLICATION NUMBER: *PCT/US06/32531*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US60/709,869*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 629341206US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Elliott P.	DAWSON	Murfreesboro, TN
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Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

A METHOD FOR THE COLLECTION AND ISOLATION OF mRNA

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☐ Application Data Sheet. See 37 CFR 1.76

☑ Specification *Number of Pages* 7

☑ Drawing(s) *Number of Sheets* 3

☐ CD(s), Number of CDs _____

☒ Other (specify) Return Receipt Postcard

Fees Due: Filing Fee of $200 ($100 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF FILING FEES AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

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Date August 19, 2005

TYPED or PRINTED NAME David A. Farah, M.D.

REGISTRATION NO. 38,134 *(if appropriate)*

TELEPHONE (626) 796-4000

Docket Number: 16306

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)			Docket No. 16306
Serial No. To be assigned	Filing Date August 19, 2005	Examiner N/A	Group Art Unit N/A

Applicant/Inventor(s): DAWSON, Elliott P. and WOMBLE, Kristie E.

Title of Invention: A METHOD FOR THE COLLECTION AND ISOLATION OF mRNA

I hereby certify that the following correspondence:

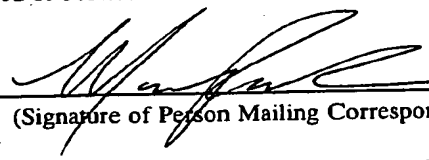
1. United States Provisional Patent Application (5 pages Disclosure, 1 page Claims and 1 page Abstract)
2. Three (3) Sheets of Drawings (Figures 1-3)
3. Provisional Application for Patent Cover Sheet (Small Entity) (1 page)
4. Return Receipt Postcard

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on: AUGUST 19, 2005.

(Date)

Marilyn Paik

(Typed or Printed Name of Person Mailing Paper or Fee)



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A METHOD FOR THE COLLECTION AND ISOLATION OF mRNA

BACKGROUND

With highly sensitive gene specific methods, such as qRT-PCR and general methods for the amplification of mRNA, minute amounts of mRNA can successfully be detected and quantified from a few cells. In many studies of human disease mRNA isolated from whole blood is commonly obtained. Frequently, special collection techniques, resources and supplies are required for collecting the relatively large volumes of whole blood needed for the subsequent isolation of mRNA. Currently available RNA blood collection tubes present limitations with the isolation methods, the time the sample can be stored at room temperature and sometimes the quality of RNA obtained. Additional factors include the complexity of recruitment and sample collection, especially for large multi-subject studies.

Therefore, there remains a need of a method of collecting mRNA that includes ease of collection, RNA stabilization and simple mRNA isolation in real world settings.

FIGURES

These and other features, aspects and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying figures where:

Figure 1 is a bar chart showing the qRT-PCR results of various housekeeping genes at various intervals over a 117-day period using the present method compared to control;

Figure 2 is a graph showing real-time PCR standard curves of the 18S gene using 10-fold serial dilutions of commercially available cDNA; and

Figure 3 is a graph showing 18S gene results for test zones obtained day 1 through day 117.

DESCRIPTION

According to one embodiment of the present invention, there is provided a method for the isolation of mRNA, and for downstream analysis from finger-stick whole blood on an

absorbent matrix. Using the present method, adequate amounts of mRNA suitable for downstream analysis, such as qRT-PCR or microarray studies, can be obtained from 10 μl of whole blood dried on an absorbent matrix in conjunction with a preservation buffer, which can be stored at room temperature over an extended period of time. The method will now be disclosed in greater detail.

In one embodiment, the present invention is a method for the isolation of mRNA from a patient, and for analysis of the mRNA. In one embodiment, the method comprises, first, obtaining whole blood from the patient from dermal or subcutaneous blood vessels, such as for example, using a finger stick, and collecting the whole blood into a suitable vessel such as a pipette tip collection or capillary tube.

Next, the whole blood is applied onto one or more one zone on an absorbent matrix containing lysing and stabilizing agents to prevent RNA degradation. Alternately, the blood from the whole blood applied directly onto the one or more than one zone on an absorbent matrix, rather than being collected into a suitable vessel. Collecting the whole blood into a suitable vessel before applying the whole blood to the absorbent matrix, however, helps assure that a fixed amount of whole blood is applied, as will be understood by those with skill in the art with reference to this disclosure.

Suitable absorbent matrices were made, for example, from 20 micron cellulose Sigmacell Type 101 (Sigma, St. Louis, MO US) particles suspended in water and formed into a paste. The paste was then applied to each of 10 holes in a plastic rectangular stick and allowed to air dry at 50°C for 2 hours. The lysis preservation buffer comprised an aqueous solution of 1% Sodium Dodecyl Sulfate, 10 mM EDTA, 10 mM MOPS, 500 mM lithium chloride and 5 mM ammonium salt of aurine tricarboxylic acid, pH 6.8 (all from Sigma). 5 μl of this lysis preservation buffer was applied to each of 5 of the 10 cellulose zones of the stick and allowed to dry for 2 hours at 50°C.

Then, mRNA is eluted from each zone by eluting the whole blood samples containing the mRNA from the absorbent matrix using standard techniques, as will be understood by

those with skill in the art with reference to this disclosure. Next, the mRNA is isolated from the eluted material using standard techniques, as will be understood by those with skill in the art with reference to this disclosure.

By way of example, the above steps were performed as follows. After application of 10 μl of whole blood to each zone of the absorbent matrix, the blood was allowed to absorb and to visibly dry. Control and test blood zones were then punched from the stick into individual tubes using a small plastic dowel for each of the successive time points. The punches for the first three time points were stored at -80°C until they could be processed. The punches were each processed by adding 100 μl of the above lysis preservation buffer to each, and completely dispersing the cellulose punches containing the applied blood into this solution. Then, 5 μl of poly-dT paramagnetic beads (Dynabeads, Dynal Biotech, L.L.C., Brown Deer, WI US) was added to the suspension of dispersed blood spots or punches and was allowed to incubate at room temperature for 4 minutes with repeated mixing by inversion. Paramagnetic beads were separated from the mixture components by inversion of the capped tubes containing the mixture and application of a magnet to the cap of the inverted tube for about 2 minutes. Next, the tubes were placed upright, allowing the liquid phase to drain into the tube while the magnet applied to the cap retained the mRNAs captured by the poly-dT paramagnetic beads. Each cap was then transferred to a fresh tube containing Wash Buffer A (Dynal Dynal Biotech). Then, the magnet was withdrawn from the cap, and the beads and wash buffer were mixed and incubated for about 2 minutes at room temperature. The cap transfer and incubation steps were repeated as described above using 2 fresh tubes of 200 μl each of Wash Buffer B (Dynal Dynal Biotech). The mRNAs were eluted from the beads by the addition of 10 μl 10 mM Tris-HCl pH 7.5 (Dynal Dynal Biotech) and heating to 60°C for 5 minutes followed by centrifugation at 5000xg to pellet the beads. The supernatant containing the mRNA was transferred to a fresh tube and stored at -80°C until processing to produce cDNA and qRT-PCR by standard methods known in the art.

In one embodiment, the method further comprises quantifying the isolated mRNA using

standard techniques, as will be understood by those with skill in the art with reference to this disclosure. In another embodiment, the method further comprises performing expression analysis on the isolated mRNA using standard techniques, as will be understood by those with skill in the art with reference to this disclosure. In a preferred embodiment, the expression analysis is qRT-PCR.

By way of example, the method for the isolation of mRNA, and for downstream analysis from finger-stick whole blood on an absorbent matrix according to the present invention was performed as follows. First, 10 μl whole blood was collected using a finger stick and collection of the whole blood into a pipette tip collection or capillary tube. Next, the whole blood was placed on multiple absorbent matrix zones containing lysing and stabilizing agents to prevent RNA degradation.

Then, mRNA isolation was completed for each zone by eluting the sample from the absorbent matrix, isolation of mRNA using oligo(dT) magnetic bead techniques and elution of mRNA in a stabilizing buffer. Next, the isolated mRNA was quantified using a Nanodrop® spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE US).

Then, expression analysis was performed on the isolated mRNA by qRT-PCR using TaqMan® gene expression assays (Applied Biosystems; Foster City, CA US) for a set of high, medium and low expression housekeeping genes (18S, GAPDH, GUSb, PGK, TBP). Next, the mRNA was isolated from sample replicate matrix zones at successive intervals extending to 117 days.

Referring now to Figure 1, Figure 2 and Figure 3, there are shown respectively, a bar chart showing the qRT-PCR results of various housekeeping genes at various intervals over a 117-day period using the present method compared to control (Figure 1); a graph showing real-time PCR standard curves of the 18S gene using 10-fold serial dilutions of commercially available cDNA (from Princeton BioMeditech Corporation, Princeton, NJ US). Day 20 showed no detectable expression of the PGK gene and no time point showed detectable expression of GUSb or TBP. (Figure 2); and a graph showing 18S gene results for test zones

obtained day 1 through day 117 (Figure 3). As can be seen, the expression levels determined from the qRT-PCR panel of housekeeping genes remain relatively consistent across time points out to the 117-day limit tested, even when stored at ambient conditions in a sealed container. The results were comparable to those from mRNA obtained from commercially available RNA blood tubes and isolation methods that require using much larger quantities of whole blood and elaborate collection and processing protocols.

Therefore, using the present method, the quality and quantity of mRNA required for qRT-PCR and amplification can successfully be obtained from a finger-stick collection of whole blood on an absorbent matrix. This is particularly useful in circumstances in which peripheral blood is suitable for expression analysis but where full scale collections are logistically difficult and or costly.

According to another embodiment of the present invention, there is provided a method of detecting the presence of specific mRNA from a patient. The method comprises, first performing the method for the isolation of mRNA from a patient, and for analysis of the mRNA according to the present invention. Next, the isolated mRNA is identified using standard techniques, as will be understood by those with skill in the art with reference to this disclosure. Then, the presence or absence of the specific mRNA is determined by reference to the mRNA identified.

Although the present invention has been discussed in considerable detail with reference to certain preferred embodiments, other embodiments are possible. Therefore, the scope of the appended claims should not be limited to the description of preferred embodiments contained in this disclosure. All references cited herein are incorporated by reference to their entirety.

WHAT IS CLAIMED IS:

1. A method for the collection and isolation of mRNA as disclosed in this disclosure.

2. A method of detecting the presence of specific mRNA from a patient as disclosed in this disclosure.

ABSTRACT

A method for the collection and isolation of mRNA. A method of detecting the presence of specific mRNA from a patient.

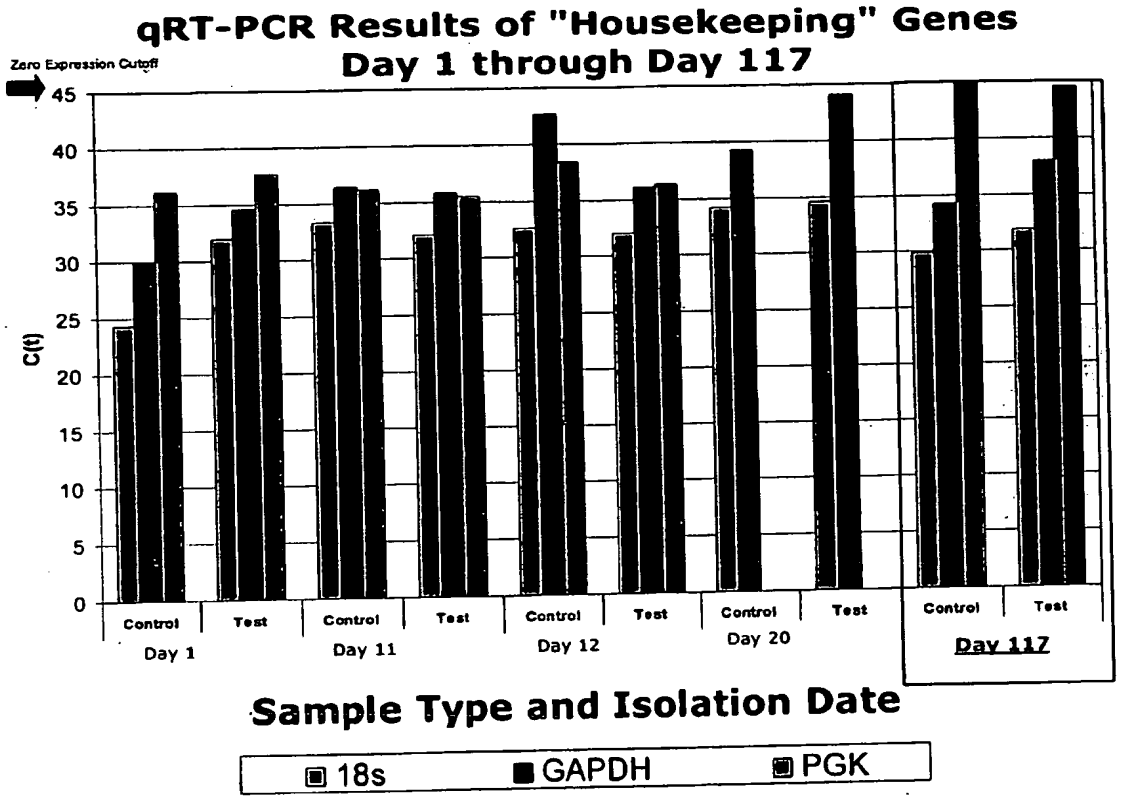
qRT-PCR Results of "Housekeeping" Genes
Day 1 through Day 117
Zero Expression Cutoff
45
40
35
30
25
20
15
10
5
0
C(t)
Control
Test
Day 1
Day 11
Day 12
Day 20
Day 117
Sample Type and Isolation Date
18s
GAPDH
PGK

FIG. 1

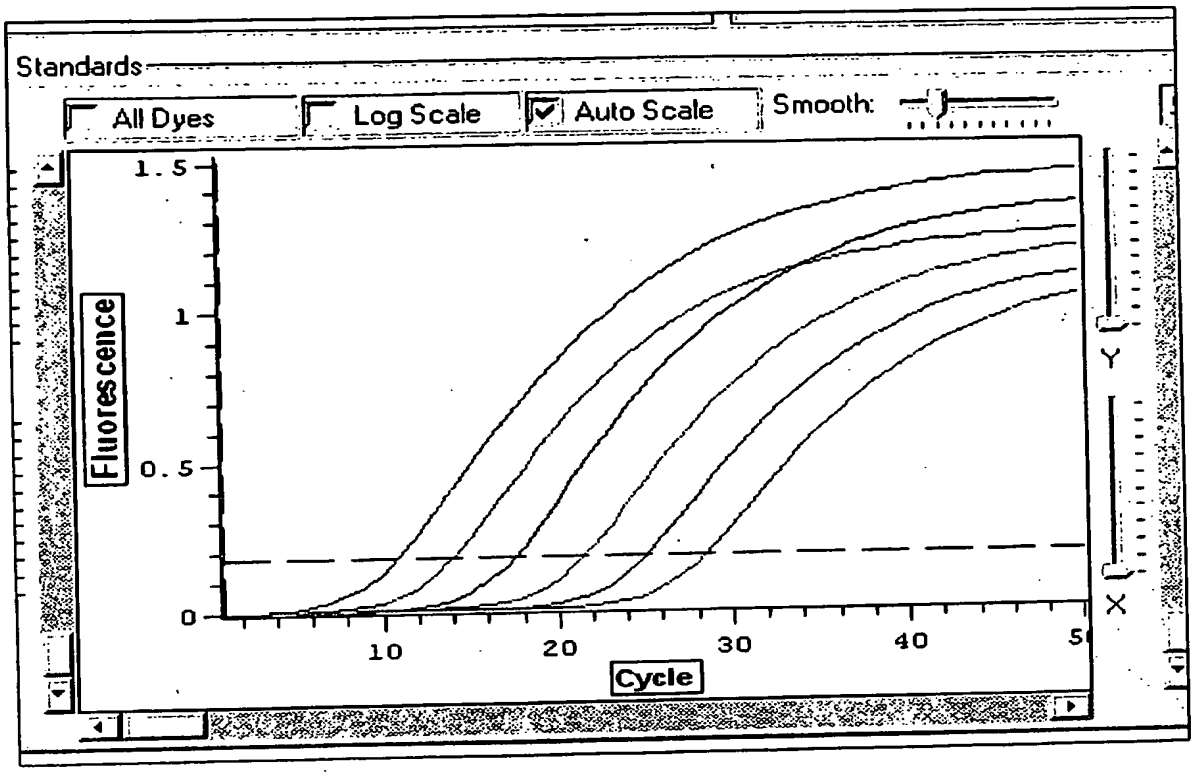
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FIG. 2

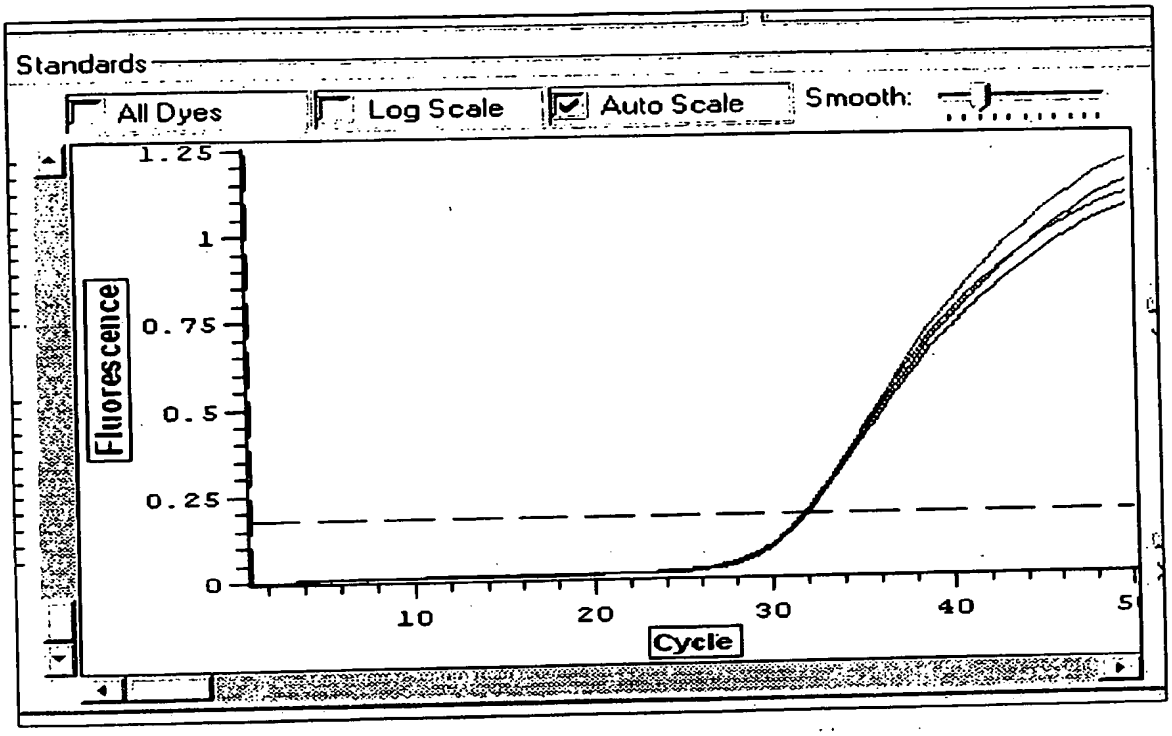
Standards
All Dyes
Log Scale
Auto Scale
Smooth:
Fluorescence
1.25
1
0.75
0.5
0.25
0
10
20
30
40
Cycle

FIG. 3